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Complete genome sequence of an Iranian isolate of Potato virus X from the legume plant *Pisum sativum*

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The natural host range of *Potato virus X* (PVX) has so far been restricted to the families *Amaranthaceae*, *Cruciferae*, and *Solanaceae*. Here we provide evidence that in Iran PVX also occurs on pea (*Pisum sativum*), a legume plant. To understand the molecular basis for this expansion of the virus host range, we sequenced a complete RNA genome of the Iranian isolate of PVX (PVX-Iran) and compared it with the genomes of 14 known PVX isolates. The PVX-Iran nucleotide sequence shares 95.4–96.7% identity with the Eurasian isolates and 77.2–78.0% with the South American ones and, like all other isolates, codes for RNA-dependent RNA-polymerase (RdRP), triple gene block proteins (TGBp) 1, 2, and 3, and coat protein (CP). At the amino acid level, PVX-Iran is most closely related to the Korean isolate (PVX-KR) sharing 98.1–99.2% identity and 98.6–100% similarity. Further inspection of PVX-Iran revealed several unique amino acid substitutions that do not occur in any other isolate, most notably A542 in RdRP, T10 in TGBp3, and T24 in CP, which individually or in combination could have contributed to breaking pea resistance to PVX.

Potato virus X (PVX) is the type member of genus *Potexvirus* in the family *Flexiviridae*. Its capped and polyadenylated, single-stranded RNA(+) genome, 6432–6435 nt in length [excluding the poly(A) tail], which is encapsidated in flexuous filamentous virions, contains five conserved ORFs. ORF1 codes for RNA-dependent RNA polymerase (RdRP) of 166 KDa, involved in genome replication and production of three subgenomic RNAs. The overlapping ORFs 2, 3, and 4 code for triple gene block proteins (TGBp) of 25, 12, and 8 kDa, respectively, all required for cell-to-cell movement. In addition, TGBp1 is a suppressor of plant defense based on RNA silencing [1]. ORF 5 encodes the coat protein (CP) of 27 KDa, which in addition to building the virions and aiding to cell-to-cell movement can elicit hypersensitive response in plants carrying specific resistance genes (for recent review on PVX, see [14]).

The natural host range of PVX has so far included plant species of the families *Amaranthaceae*, *Cruciferae*, and *Solanaceae*. Using serological methods, electron microscopy, and RT-PCR we detected PVX on pea (*Pisum sativum*; the family *Leguminosae* or *Fabaceae*) in Iran [4, 5]; unpublished data). Among other viruses identified on symptomatic pea plants in several commercial fields of the Teheran province during 2002 and 2004, the incidence of PVX systemic infection was the highest (67%). The PVX symptoms observed in naturally-infected pea plants included stunting and wilting as well as premature death of young seedlings. To our knowledge, this is the first case of natural occurrence of PVX on pea, though PVX was reported, following experimental inoculation, to cause systemic infection on crimsom clover (*Trifolium incarnatum*) but not other legume plants tested [15] and local necrotic lesions on some cultivars of pea [3]. The latter finding suggests that pea and other legumes carry gene(s) conferring resistance to PVX, which in some cases trigger hypersensitive response. As a first step to understanding the

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Table 1 Percentage of nucleotide identity and amino acid identity/similarity of PVX-Iran to other PVX isolates

PVX isolate	Accession no.	Whole genome 6435 nt	5′ UTR 84 nt	RdRP 4371 nt 1457 aa	TGB1 681 nt 227 aa	TGB2 348 nt 116 aa	TGB3 213 nt 71 aa	CP 714 nt 238 aa	3′ UTR 82 nt
PVX-KR Korea	AF373782	96.7	96.4	96.3	97.2	98.0	97.2	97.2	100.0
				98.1/99.2	99.6/100.0	99.1/100.0	98.6/98.6	99.2/99.2	
PVX-Taiwan China	AF272736	96.6	97.6	96.2	96.8	97.7	97.7	97.6	100.0
				98.4/99.1	98.2/99.6	98.3/99.1	95.8/97.2	99.2/99.2	
PVX-X3 Europe	D00344	96.5	100.0	96.1	96.9	97.7	97.2	97.2	100.0
				97.9/98.9	98.7/99.1	99.1/100.0	97.2/98.6	99.2/99.2	
PVX-FX21 China	EF423572	96.4	96.4	96.2	96.0	96.3	97.7	97.1	100.0
				98.1/98.8	98.2/99.1	99.1/100.0	97.2/97.2	99.2/99.2	
PVX-Moscow Russia	M72416 M37458, X05198	96.2	97.6	95.9	96.8	96.6	97.7	96.1	100.0
				98.2/99.0	98.2/99.6	99.1/100.0	97.2/97.2	97.5/99.2	
PVX-OS Japan	AB056718	96.2	97.6	95.6	96.5	98.0	98.6	97.3	100.0
				98.3/99.1	97.8/99.1	99.1/100.0	98.6/98.6	98.7/98.7	
PVX-UK3 UK	M95516	96.0	96.4	95.6	95.9	97.7	95.8	97.2	100.0
				97.9/98.7	97.8/98.2	99.1/100.0	98.6/98.6	98.7/98.7	
PVX-BS Japan	AB056719	95.8	98.8	95.3	96.2	96.6	98.1	95.9	100.0
				97.9/98.9	98.2/99.1	97.4/100.0	97.2/97.2	97.5/97.9	
PVX-Roth1 UK	AF111193	95.6	100.0	95.3	95.0	97.7	96.7	95.9	98.6
				97.7/98.6	98.7/100.0	99.1/100.0	98.6/98.6	98.3/98.7	
PVX-Tula Russia	EU021215	95.4	95.2	95.3	95.6	97.1	97.2	95.0	100.0
				98.1/99.0	98.2/100.0	99.1/100.0	98.6/98.6	97.9/98.7	
PVX-HB South America	X72214	78.0	88.1	77.5	77.3	82.1	71.9	78.7	95.8
				90.0/94.5	89.9/94.3	88.8/94.8	70.8/79.2	88.2/93.3	
PVX-HBh South America	Z23256	77.9	88.1	77.4	77.0	82.6	71.4	79.2	95.8
				90.0/94.5	88.1/93.0	89.7/95.7	69.4/79.2	89.5/94.5	
PVX-CP South America	X55802	77.3	88.1	76.6	77.7	79.1	72.7	79.6	95.8
				89.3/93.8	89.9/93.0	88.8/94.8	71.8/81.7	89.1/94.1	
PVX-CP4 South America	AF172259	77.2	88.1	76.6	77.6	79.6	70.6	78.9	95.8
				89.9/94.2	89.0/92.5	89.7/94.8	70.4/80.3	89.9/95.0	

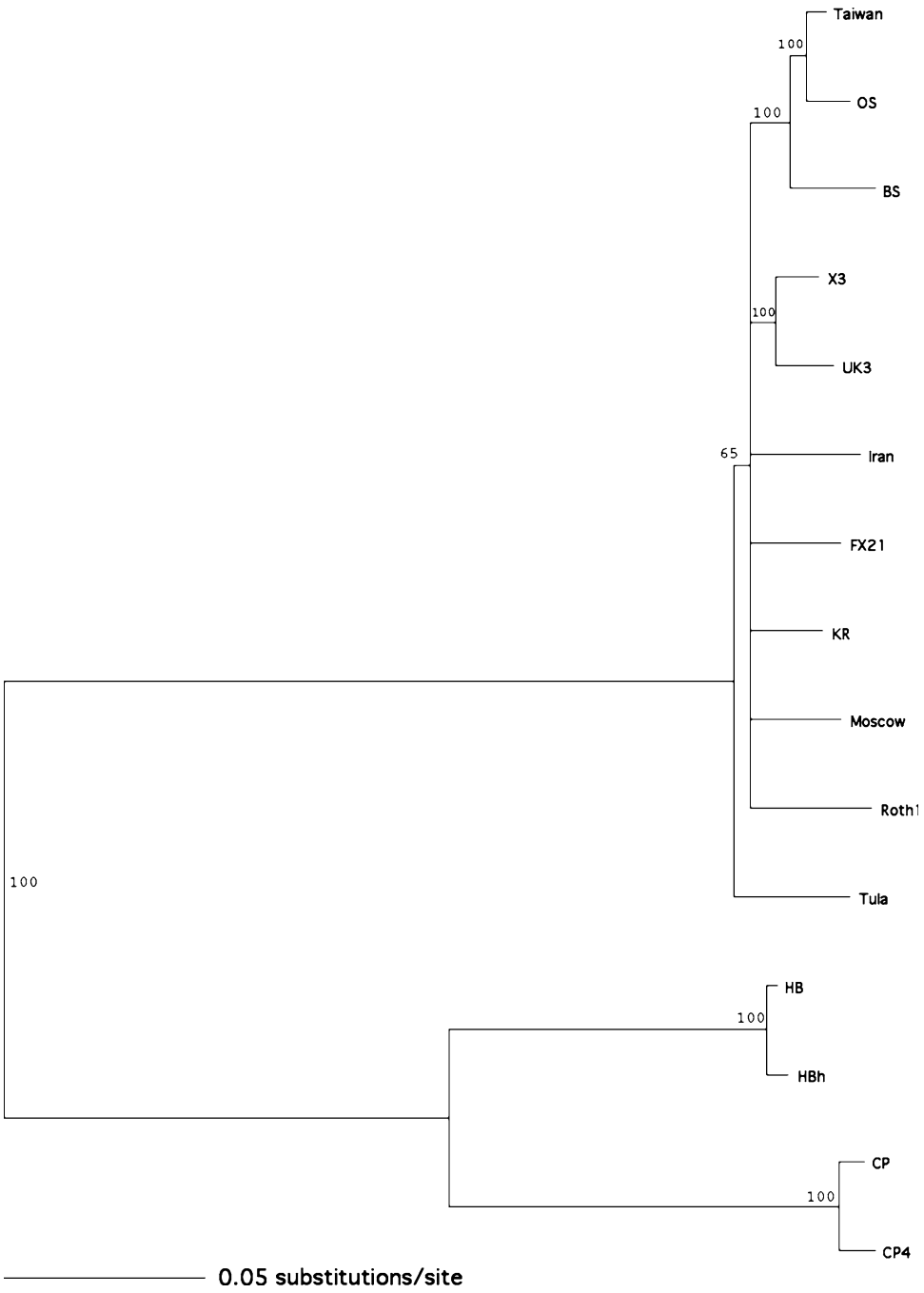

Fig. 1 A Neighbor Joining tree showing the genetic distances between the nucleotide sequences of 15 isolates of PVX. Analysis was performed with PAUP 4.0 (Phylogenetic Analysis Using Parsimony, Version 4; Sinauer Associates, Sunderland, Massachusetts). The tree was rooted using four South American isolates (HB, HBh, CP and CP4) as an outgroup. The bootstrap values are indicated. The cutoff for collapsing the branches was 60%

molecular basis for breaking pea resistance to PVX, we sequenced a complete genome of the Iranian isolate of PVX (PVX-Iran) and performed its phylogenetic analysis.

The complete genomes of fourteen PVX isolates have been reported (Table 1; [6–11, 13, 16]. To sequence a complete RNA genome of PVX-Iran we designed RT-PCR primers (Table S1), which would anneal to the regions conserved in the sequenced PVX isolates.

PVX-Iran was purified after three successive single lesion transfers on *Gompherena globosa* and one systemic infection on *Nicotiana glutinosa* and used for the host range studies with various plant species [4, 5] including *Nicotiana benthamiana*. A back passage of the virus isolate to pea resulted in leaf wilting followed by death of infected plants, indicating that the pea-infecting phenotype was stable over the passages. Total RNA was isolated from systemically-infected leaves of *N. benthamiana* 14 days post-inoculation using an RNeasy plant mini kit (Qiagen) and transcribed by SuperScript III reverse transcriptase (Invitrogen) with the RT primer (Table S1) complementary

to the absolutely conserved PVX 3′-terminal sequence preceding poly(A). The resulting viral cDNA was used for consecutive PCR reactions with Vent polymerase (BioLabs) to amplify eight overlapping fragments, ~0.8–1.2 kbp in length, covering the entire genome of PVX-Iran. Each PCR product was gel-purified and sequenced in both orientations using the corresponding forward and reverse PCR primers (Table S1). The assembled genome sequence of PVX-Iran (GenBank accession no. FJ461343) was complete with exception of the 5′- and the 3′-terminal sequences of 24 and 28 nts, respectively (corresponding to the terminal PCR primers; Table S1), in which some nucleotide substitution(s) could not be excluded. The comparison of PVX-Iran with the 14 other PVX isolates at the nucleotide and amino acid levels were done by the Needleman–Wunsch global pair-wise alignment and the CLUSTAL W multiple alignment programs (http://bioweb2.pasteur.fr/intro-en.html) and the results are shown in Table 1 and Supplementary Data. A Neighbor Joining tree showing the genetic distances between the 15 isolates is shown in Fig. 1.

Phylogenetically, PVX-Iran falls within the Eurasian (EA) group of PVX isolates and shows 95.4–96.7% identity at the whole genome nucleotide level to 10 other members of the group described previously [11, 16]. It is more distantly related to 4 isolates of the South American (SA) group (72.2–78.0% identity) (Table 1 and Fig. 1). PVX-Iran contains five conserved ORFs for RdRP, TGBp1, TGBp2, TGBp3, and CP, which are identical in position and length to those of all the other EA isolates. At the amino acid level, PVX-Iran is most closely related to the Korean isolate (PVX-KR) showing 98.1–99.2% identity and 98.6–100% similarity and least related to the PVX-CP4 isolate from the SA group showing 70.4–89.9% identity and 80.3–95.0% similarity, depending on the protein (Table 1). Inspection of the multiple protein alignments (Supplementary Data) revealed several unique amino acid substitutions present in PVX-Iran but not in any other EA or SA isolate. These include E134 (D in other EA isolates; H in SA isolates), A490 (V/I/G in EA; P in SA), A542 (E/D in EA; T in SA), A613 (V in all others), I651 (M in all others) and V811 (I in all others) in RdRP; D177 (E in all others) in TGBp1; T10 (I in all others) in TGBp3; and T24 (V in PVX-OS; A in all others) in CP. In TGBp2 no unique amino acid was found, except that PVX-Iran and PVX-Tula share H98, while other EA isolates have Y and SA isolates have C at this position. Based on amino acid similarity scores, the RdRP A490, the TGBp3 T10 and the CP T24 are most dissimilar at the corresponding positions. The PVX coat protein is one of the principle determinants of the outcome of interactions between a virus isolate and potato cultivars carrying distinct resistance genes, leading to resistance or systemic infection [2, 9, 12]. Our further comparison of the CP amino acid sequences of PVX-Iran and 36 other isolates found in the Genbank [16] confirmed that T24 is a unique signature of the PVX-Iran coat protein (Supplementary Data).

We propose that the amino acid substitutions in CP and/or other viral proteins could have contributed to breaking pea resistance to PVX. However, we cannot exclude that changes at the nucleotide level, especially in untranslated regions, could have also played a role. Thus, future studies should address which of the changes in the PVX genome are responsible the expansion of its host range to a legume plant.

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